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# Antibodies and coinfection drive variation in nematode burdens in wild mice



Melanie Clerc<sup>a,b,\*</sup>, Godefroy Devevey<sup>a</sup>, Andy Fenton<sup>c</sup>, Amy B. Pedersen<sup>a</sup>

<sup>a</sup> Institute of Evolutionary Biology and Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3FL, UK

<sup>b</sup> MRC Centre for Inflammation Research, The Queen's Medical Research Institute, University of Edinburgh, EH16 4TJ, UK

<sup>c</sup> Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK

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## ABSTRACT

Coinfections with parasitic helminths and microparasites are highly common in nature and can lead to complex within-host interactions between parasite species which can cause negative health outcomes for humans, and domestic and wild animals. Many of these negative health effects worsen with increasing parasite burdens. However, even though many studies have identified several key factors that determine worm burdens across various host systems, less is known about how the immune response interacts with these factors and what the consequences are for the outcome of within-host parasite interactions. We investigated two interacting gastrointestinal parasites of wild wood mice, *Heligmosomoides polygyrus* (nematode) and *Eimeria* spp. (coccidia), in order to investigate how host demographic factors, coinfection and the host's immune response affected parasite burdens and infection probability, and to determine what factors predict parasite-specific and total antibody levels. We found that antibody levels were the only factors that significantly influenced variation in both *H. polygyrus* burden and infection probability, and *Eimeria* spp. infection probability. Total faecal IgA was negatively associated with *H. polygyrus* burden and *Eimeria* spp. infection, whereas *H. polygyrus*-specific IgG1 was positively associated with *H. polygyrus* infection. We further found that the presence of *Eimeria* spp. had a negative effect on both faecal IgA and *H. polygyrus*-specific IgG1. Our results show that even in the context of natural demographic and immunological variation amongst individuals, we were able to decipher a role for the host humoral immune response in shaping the within-host interaction between *H. polygyrus* and *Eimeria* spp.

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## 1. Introduction

Parasitic helminths can negatively affect individual health and impact the population dynamics of wild animals, livestock and humans (Charlier et al., 2015; Rose et al., 2014; Weinstein and Lafferty, 2015). For example, an estimated 39 million disability-adjusted life-years (DALYs) were lost between 1993 and 1994 due to four human intestinal helminth species (*Ancylostoma duodenale*, *Necator americanus*, *Ascaris lumbricoides* and *Trichuris trichiura*) (Stephenson et al., 2000), and population crashes in wild Soay sheep, Red grouse, and wild mice have been linked to helminth infections (Gulland, 1992; Hudson et al., 1998; Pedersen and Greives, 2008). Therefore, much effort has been devoted to study the immune pathways and cell types involved in resistance to important helminth infections (Sorobetea et al., 2018). However,

most of this work has been carried out in well-controlled laboratory settings in order to minimise environmental variation and other potential confounding factors. Under natural circumstances, however, both animals and humans are rarely uniform in factors such as sex, age, behaviour, nutritional status and genetics (Charmantier and Garant, 2005; Turner et al., 2011; Nussey et al., 2012). Discrepancies between the reductionist approach of traditional immunological studies and the diversity of the real world make it difficult to extrapolate the role of many immune functions derived from controlled and homogenised laboratory studies, to predict their impact or importance for individual infection levels and health in the natural setting.

A major issue contributing to the difficulty in extrapolating from the laboratory to the field is the frequent use of immunologically naïve animals in laboratory experiments, which do not readily compare with wild animals that can face repeated and diverse parasite and pathogen challenges. This incompatibility has been highlighted by a recent study comparing the immune cell composition of laboratory-raised, pet shop and feral mice, showing that

\* Corresponding author at: MRC Centre for Inflammation Research, The Queen's Medical Research Institute, University of Edinburgh, EH16 4TJ, UK.

E-mail address: [m.clerc@ed.ac.uk](mailto:m.clerc@ed.ac.uk) (M. Clerc).

naïve laboratory mice immunologically resemble human newborns rather than adults, whilst normal immune development was found for both pet shop and wild mice (Beura et al., 2016). A recent review by Viney and Riley (2017) further emphasises the comparability issues between laboratory-raised mice and wild mice, with the immune system of the former consistently showing less activation and evidence of antigenic exposure compared with the latter across studies (Viney and Riley, 2017). The authors also point towards the stark over-representation of laboratory-based experiments compared with studies carried out in the wild (19,997 versus 26 publications in 2016; Viney and Riley, 2017). Added to that is the fact that prior or current coinfection with multiple parasite species is the norm rather than the exception (Petney and Andrews, 1998; Rigaud et al., 2010; Griffiths et al., 2011). These coinfecting parasites often interact with each other (Griffiths et al., 2014), either directly through parasite-induced toxins, tissue damage or physical interference, or indirectly through competition for shared host resources or the host immune system (Christensen et al., 1987; Cox, 2001; Pedersen and Fenton, 2007). This means that coinfection can impact an individual's ability to mount an effective immune response and control infection of a focal parasite. For example, it has been shown repeatedly that helminths cause significant morbidity/mortality when individually infecting a host, and are further associated with increased susceptibility of humans to HIV (Downs et al., 2017) and *Mycobacterium tuberculosis* (Lang and Schick, 2017). Hence, previous parasite exposure and (natural) coinfection are two key factors that can determine an individual's susceptibility to infection and the outcome of disease. However, few studies in wild animals have explored how heterogeneity in environment, host demography and coinfection affect the host's immune response (but see Ezenwa et al., 2010; Babayan et al., 2011; Pedersen and Babayan, 2011; Japp et al., 2017).

Studies measuring the immune response of naturally infected animals offer a valuable avenue to complement traditional immunology and fill the gap between evidence gathered from the laboratory and the wild. Wild wood mice (*Apodemus sylvaticus*) harbour a diverse parasite community comprising >30 different microparasite and macroparasite species with often >60% of individuals being coinfecting by two or more parasite species (Knowles et al., 2013). In the wild, we previously found that the use of a broad spectrum anthelmintic drug (Ivermectin) reduced the prevalence of the most common helminth species, *Heligmosomoides polygyrus*, by approximately 70% (Knowles et al., 2013). However, we also found that Ivermectin-treated mice showed a subsequent 15-fold increase in the intensity of the coinfecting protozoan parasite *Eimeria hungaryensis* 1–3 weeks post-treatment (Knowles et al., 2013), suggesting a strong negative interaction between the two parasites. These parasites trigger opposing arms of the immune system (Th2 in the case of *H. polygyrus*, Th1 in terms of *Eimeria* spp.), making it an ideal study system to investigate the role of the immune response in mediating this interaction, in combination with the impact of demographic variation and coinfection on the potential interrelationships between the immune system and parasite infection.

We used cross-sectional data from wild-trapped *A. sylvaticus* populations to measure factors representing host demographics, coinfection and the host's immune response. Specifically, we measured two antibodies as a simplified read-out for host immune activity from each individual caught. From blood serum, we measured titres of *H. polygyrus*-specific IgG1 as an indicator of the magnitude of an anti-*H. polygyrus* immune response. This antibody is highly important in the immune response against *H. polygyrus*, as it reduces fecundity of adult female worms (Hewitson et al., 2011; Maizels et al., 2012; Reynolds et al., 2012) and correlates negatively with worm burden (Ben-Smith et al., 1999). Generally,

resistance towards *H. polygyrus* in the laboratory is associated with a strong and quickly established Th2 type immune response, with mice that also show increased Th17 and/or Treg cell activity usually being more susceptible towards (re)infection (Maizels et al., 2012). Further, we measured the concentration of total faecal IgA from host faecal extracts. IgA is the most common antibody on mucosal surfaces and its production is triggered by a multitude of microbial stimuli (pathogenic and commensal), as well as different helminth species (Fagarasan and Honjo, 2003; Macpherson et al., 2012). It has previously been shown that IgA from caecal contents of chicken had high anti-schizont and sporozoite-neutralising activities with regard to infection with *Eimeria maxima* (Davis et al., 1978; Trees et al., 1989). Further, mice that were able to quickly clear *H. polygyrus* infections also had higher levels of parasite-specific IgA than slow-resolving mice (Ben-Smith et al., 1999; Behnke et al., 2003). As we measured total levels of IgA rather than parasite-specific levels, we consider the read-out of IgA to represent overall immune activation, i.e. high total IgA levels suggest current infection and/or accumulation of previous parasitic exposures with both *H. polygyrus* and *Eimeria* spp. Even though there are many other immune components involved during infection with either parasite (e.g. transcription factors, cytokines, different cell types), antibodies represent one of the downstream consequences of these immune cascades, and in the case of helminth infections, total faecal IgA levels have been shown to correlate with parasite-specific IgA levels in wild Soay sheep (Watt et al., 2016). Further, measuring these two immune effectors has proven to be feasible given sample collection under field conditions.

It is clear that the cause-and-effect relationships between immune read-outs and parasitic infections are often not as straightforward to define in the wild compared with controlled laboratory infection experiments. This makes it difficult to distinguish between immune effectors (i.e. is a cell type/molecule associated with reducing parasite load or clearance) and immune markers (i.e. is a cell type/molecule stimulated by the presence of an infection), both of which are not mutually exclusive. Here we aimed to analyse both possibilities, by first testing how host demographic factors, coinfection and the host's immune response drove variation in parasite burdens and infection probability, and then, in turn, investigating which factors predict parasite-specific and total antibody levels. Combining these two types of approaches allowed us to gain vital insights into the likelihood for either of the two possibilities, thereby allowing an unbiased approach to understanding cause-and-effect relationships between immune markers and parasite infections in the wild.

## 2. Materials and methods

### 2.1. Field experiment

In October 2013, wild wood mice (*A. sylvaticus*) were trapped in a mixed woodland in northwestern England (53°16'12.0"N, 3°01'48"W). Trapping took place over the course of five consecutive days, with 96 Sherman Live Traps (H.B. Sherman 5.08 × 6.35 × 16.51 cm folding traps, Tallahassee, FL, USA) being baited with grains, carrots and cotton bedding, and set up each day at dusk in three longitudinal transects. Each transect had 16 trapping stations (two traps per station), which were 10 m apart from each other. Transects were laid out parallel to each other, with 10 m space between transects. Traps were checked the following morning and we measured the following parameters of all non-gravid (pregnant or lactating) females and all males: body mass (g), body length (mm), sex, body condition (vertebral column and pelvic bones fat reserves, each scored from 1 to 5: 1 being bones sharp and easily distinguishable with no pressure applied, and 5 being

bones barely distinguishable even when pressure applied (Burthe et al., 2006). We also assessed the reproductive status of each animal, with females grouped into either non-reproductive, perforated, pregnant or lactating, and males grouped into either non-reproductive (testes not descended), reproductively active (testes descended) or scrotal. We further checked for the presence and burden of ectoparasites (i.e. fleas and ticks) using standard methods (Withenshaw et al., 2016).

Mice were culled via cervical dislocation and dissected the same day they were trapped. For each mouse, we collected a terminal blood sample via cardiac puncture following culling. The blood was centrifuged for 10 min at 11,800g and red blood cells and serum were stored separately at  $-20^{\circ}\text{C}$ . We further removed both eyes from each mouse and stored them in pairs in 10% v/v formalin for approximately 8 weeks before dissecting out the lenses. They were subsequently dried at  $56^{\circ}\text{C}$  overnight and weighted in pairs to the nearest milligram. We used this metric as a quantitative proxy for host age, since previous studies have shown strong correlations between combined eye lens weight and age in various species of small mammals in both the laboratory and field (Rowe et al., 1985; Tanikawa, 1993; Burlet et al., 2010). Using this metric, Friberg et al. (2011) were able to discriminate between “young-of-the-year” wood mice, which had a combined eye lens weight  $<20$  mg, and “over-wintered” wood mice, which had a combined eye lens weight  $>20$  mg.

## 2.2. Intestinal parasite analysis

Shortly after the mice were culled, their small intestines, colons and caeca were removed and checked for the presence and burden of gastrointestinal helminthes using a dissection microscope. Worms were counted, sexed (only possible for *H. polygyrus*) and identified to the finest taxonomic level possible. Individuals of *Syphacia* spp. and *Aspicularis* spp. were grouped together as ‘pinworms’ due to their similar appearance, life cycle and immunological effect on the host (Taffs, 1976; Grear and Hudson, 2011). To assess the burden of *Eimeria* spp. parasites, we collected a faecal sample for each mouse from the previously sterilised trap it was caught in. Samples were stored in 10% v/v formalin at  $4^{\circ}\text{C}$  and saturated salt flotation (Dryden et al., 2005) was used to separate parasite transmission stages from faecal material. In short, faecal pellets were homogenised and poured into a faecalizer (“Fecal Ova Float”, KV supply, United States, Cat.no. 81253). These were filled to the top with saturated salt solution until a meniscus had formed. A glass cover slip was placed on top of the faecalizer and let stand for 15 min. During this time, parasite transmission stages floated to the top and attached to the cover slip. After removing the cover slip from the faecalizer, it was placed on a glass slide and scanned for the presence and burden of parasite transmission stages using a microscope at either  $10\times$  or  $40\times$  magnification. Parasite transmission stage counts were standardised at eggs/oocysts per 1 g of faeces. Additionally, 2–3 pellets were dry-frozen at  $-80^{\circ}\text{C}$  for later IgA ELISAs.

## 2.3. Immunological methods

We used ELISA assays to quantify antibody titres of *H. polygyrus*-specific IgG1 from blood sera (Hewitson et al., 2011), and total faecal IgA concentrations from faecal extracts of *A. sylvaticus* (Watt et al., 2016). The amount of parasite-specific antibodies in serum samples, such as *H. polygyrus*-specific IgG1, can only be measured relative to the background absorbance levels of the ELISA plate. Therefore, the values for *H. polygyrus*-specific IgG1 are measured as “titres”, rather than actual concentrations, i.e. a titre of 12,800 means that serum dilutions of 1 in 12,800 still showed absorbance greater than the mean absorbance of the blank

plate plus 3 S.D. This would be considered a high titre for *H. polygyrus*-specific IgG1, whereas a titre of 100 (positive signal from a serum dilution of 1 in 100) would be considered low. On the other hand, the concentration for total antibodies, such as faecal IgA, can be accurately calculated because the reference sample in this case is a synthetically manufactured standard antibody that contains a known quantity of IgA. We refer to both *H. polygyrus*-specific IgG1 titres and IgA concentrations as “antibody levels” in the following text.

All reagents used for immunological assays were specific to *Mus musculus*, as there are currently no reagents specific for non-standard laboratory mice available. Efforts have been taken to optimise and adjust their performance when used with serum/faecal extracts from *Apodemus sylvaticus*. Further, positive controls in the form of sera/faecal extracts from laboratory mice artificially infected with *Heligmosomoides bakeri* (closely related to *H. polygyrus* and used in most laboratory experiments) have been used in all assays.

To measure *H. polygyrus*-specific IgG1, we coated plates (Nunc™ MicroWell™ 96-Well Microplates ThermoFisher Scientific, United Kingdom) with *H. polygyrus* excretory-secretory antigen collected from adult worms (HES; supplied by R.M. Maizels, Wellcome Trust Centre for Molecular Parasitology, University of Glasgow, United Kingdom 1.0 µg/ml) diluted in carbonate buffer overnight at  $4^{\circ}\text{C}$ . Non-specific binding sites were blocked with Tris-buffered saline (TBS) containing 4% w/v BSA at  $37^{\circ}\text{C}$  for 2 h. Twofold serial dilutions of serum samples were prepared containing TBS-1% w/v BSA, starting at 1:100. A combined serum sample from multiple laboratory *M. musculus* that were artificially infected with *H. polygyrus* was added to each plate as a positive control (supplied by R. M. Maizels). After plates were washed with TBS-0.1% Tween 20 v/v, sample dilutions were added to the plates (50 µl per well) and incubated overnight at  $4^{\circ}\text{C}$ . After washing, 50 µl of goat anti-mouse IgG1-horse radish peroxidase (HRP) detection antibody (Southern Biotech, Lot J6908-MC69), diluted 1:2,000 in TBS-1% w/v BSA were added to each well and incubated at  $37^{\circ}\text{C}$  for 1 h in the dark. Plates were washed four times with TBS-Tween 20 and twice with  $\text{dH}_2\text{O}$ , before 50 µl of Tetramethylbenzidine (TMB) solution were added to each well. Plates were immediately covered to allow the enzymatic reaction to develop for 7 min and the reaction was stopped with 50 µl of 0.18 M sulphuric acid. Absorbance was measured at 450 nm. Cut-off values were calculated per plate as the mean absorbance of blank wells plus three times the S.D. of blank wells. The sample titre was determined as the denominator of the lowest sample dilution step that showed absorbance greater than the cut-off value.

For the faecal IgA ELISA, faecal extracts were prepared by soaking faecal pellets in a 3:1 vol of protease inhibitor solution (Complete Mini Protease Inhibitor Tablets, Roche, Switzerland Cat No.: 11836153001). The extraction was incubated for 1 h at room temperature, after which samples were centrifuged at 11,800g for 5 min and the supernatant containing IgA removed. ELISA plates were coated with unlabelled goat anti-mouse IgA (Southern Biotech, United States, Lot H7912-S233, 2 µg/ml) diluted in carbonate buffer overnight at  $4^{\circ}\text{C}$ . Non-specific binding sites were blocked with TBS containing 4% w/v BSA at  $37^{\circ}\text{C}$  for 2 h. Faecal extracts were diluted 1:100 in cluster tubes containing TBS-1% w/v BSA and added to the plates as triplicates, 50 µl per well. Two twofold serial dilutions of standard antibody (Purified mouse IgA,  $\kappa$  isotype control, BD Pharmingen, United States Lot 3039828) at 50 µl per well were added to each plate. Plates were incubated overnight at  $4^{\circ}\text{C}$  and then after washing, 50 µl of goat anti-mouse IgA-HRP (Southern Biotech, United States Lot G4512-V522D) diluted 1:4000 in TBS-1% w/v BSA were added to each well and incubated at  $37^{\circ}\text{C}$  for 1 h in the dark. Plates were washed four times with TBS-Tween and twice with  $\text{dH}_2\text{O}$ , before 50 µl of TMB solution



were added to each well and plates were immediately covered to allow the enzymatic reaction to develop for 7 mins. The reaction was then stopped with 50  $\mu$ l of 0.18 M sulphuric acid and absorbance at 450 nm was measured. Sample concentrations of total faecal IgA were determined by fitting four-parameter logistic regression to standard curves using online software ([www.elisa-analysis.com](http://www.elisa-analysis.com), ©Copyright 2012 Elisakit.com Pty Ltd. Australia).

#### 2.4. Statistical analysis

All statistical analyses were performed using R software version 3.2.2 (R Development Core Team (2013), [www.r-project.org](http://www.r-project.org)). As stated earlier, since we used a cross-sectional, destructive sampling design, this makes it difficult to disentangle the cause-and-effect relationships from associations between immunological measurements and parasitological status (i.e., whether antibody levels are a marker or a driver of parasite burden). Therefore, we analysed associations between parasite and immunological metrics in both directions and discuss the implications of this analysis further in the Discussion.

##### 2.4.1. Do antibody levels predict parasite infection and burden?

First, we asked whether antibody levels, host demographic factors and coinfection were predictive of natural burdens and infection risk of the interacting parasites *H. polygyrus* and *Eimeria* spp., and of pinworms, which have not been shown to interact with either of the other parasite species. We defined infection risk as the probability of being infected with either parasite upon capture. The burden data for all parasites were characterised by a large proportion of uninfected animals, and the underlying processes generating these zero burdens, i.e. why an animal is uninfected whilst others become infected, are likely to be different from the processes that generate variation within the parasite burdens of infected animals (Poulin, 2013). For example, a lack of exposure and/or resistance can account for an animal becoming infected, whereas external and/or internal trade-offs, i.e. resource limitation and/or immune conflict due to coinfection, can account for the variation in worm burden amongst infected individuals. This has been observed in many human and animal populations, with the consequence of modelling techniques being developed to account for these situations (e.g. zero-adjusted mixture count models, see Chipeta et al., 2014). To account for this, we modelled infection probabilities and intensities in infected animals separately. *Heligmosomoides polygyrus* and pinworm infections were both integer variables, hence we used the hurdle() function from the “pscl” package in R. This function simultaneously fits a negative binomial generalised linear model (GLM) to the zero versus non-zero part of the data (infection probabilities) and a poisson GLM to the continuous positive part of the data (infection intensities). Further, for the non-integer variable *Eimeria* spp. infections (log-transformed oocyst counts), we individually fitted a binomial GLM and a poisson GLM in line with the modelling approach adapted above. The predictor variables tested in all models were host sex (two-level factor, male versus female), body mass (continuous), age (eye lens weight, continuous), *H. polygyrus*-specific IgG1 titre (continuous), total faecal IgA concentration (continuous) and ectoparasite infection status (two-level factor, infected versus uninfected). If *H. polygyrus* was the response, we also included *Eimeria* spp. infection status (two-level factor, infected versus uninfected), pinworm infection status (two-level factor, infected versus uninfected) and interactions between *Eimeria* spp. and IgG1, and *Eimeria* spp. and IgA, to account for the effect of *Eimeria* spp. on the host's immune response. When *Eimeria* spp. was the response, we also included *H. polygyrus* infection status (two-level factor, infected versus uninfected). Best fitting models were estimated by stepwise backward exclusion of non-significant terms (cut-off *P* value was 0.05), either manually

or using the stepAIC() function from the “MASS” package (<http://www.stats.ox.ac.uk/pub/MASS4/>). To determine whether reduction of a model increased or decreased model fit, we performed log-likelihood ratio tests on nested models, as well as considering the change in Akaike's Information Criterion (AIC) upon reduction. A non-significant log-likelihood ratio test and a difference in Akaike's Information Criterion ( $\Delta$ AIC) between two nested models >2 was considered an improvement in model fit.

##### 2.4.2. Does parasite infection predict antibody levels?

Second, we asked whether host demographic factors and parasitic infections were predictive of IgG1 (log-transformed) and IgA (square-root transformed) levels. To test for this, we fitted a GLM with Poisson errors to each of these two response variables. We used the same predictor variables described above: body mass, host age, *Eimeria* spp. infection status, *H. polygyrus* infection status, ectoparasite infection status, pinworm burden, faecal IgA concentration (when *H. polygyrus*-specific IgG1 was the response) or *H. polygyrus*-specific IgG1 titres (when faecal IgA was the response). Best fitting models were estimated by stepwise backward exclusion of non-significant terms (cut-off *P* value was 0.05), beginning with interaction terms and comparing models using log-likelihood ratio tests.

#### 2.5. Data accessibility

Data are available on Dryad (doi: <https://doi.org/10.5061/dryad.hv8qm00>).

### 3. Results

In total, we analysed 54 *A. sylvaticus* (31 males and 23 females). Eye lens weights ranged from 10.7 to 26.3 mg (mean =  $16.4 \pm 0.45$  S.E.), which did not differ between males and females (linear model; effect of sex:  $t = -0.16$ ,  $P = 0.98$ , sex  $\times$  body mass:  $t = 0.06$ ,  $P = 0.88$ ). Seven animals had eye lens weights >20 mg, suggesting that these mice were likely older, over-wintered animals from 2012 (Friberg et al., 2011). Overall, we found that mice harboured a rich parasite community: ectoparasites were common, with 72% of mice infected by ticks (mostly *Ixodes trianguliceps*), and 7% infected by one of three flea species (*Ctenophthalmus nobilis*, *Rhadinopsylla pentacantha*, *Amalaraeus penicilliger mustelae*). In the gastrointestinal tract, we found pinworms at a prevalence of 63% (34 infected animals) and *H. polygyrus* at a prevalence of 59% (32 infected animals). Further, we found 19% of mice to be infected with *Eimeria* spp. (10 animals), while 11% were coinfecting with both *H. polygyrus* and *Eimeria* spp. (six animals).

##### 3.1. Do antibody levels predict parasite infection and burden?

Total faecal IgA concentration was the only predictor of *H. polygyrus* worm burden, with higher IgA levels being associated with lower *H. polygyrus* worm counts (Table 1, Fig. 1A). Conversely, the probability of *H. polygyrus* infection was positively associated with *H. polygyrus*-specific IgG1 (Table 1, Fig. 1B). None of the covariates we tested were associated with *Eimeria* spp. burden, but *Eimeria* spp. infection probability was negatively associated with total faecal IgA concentration (Table 1, Fig. 1C). We also did not find any associations between the covariates we tested and pinworm infection probability, but did find a negative association between pinworm burden and *H. polygyrus*-specific IgG1 titre (Table 1, Fig. 1D).

**Table 1**

Results from minimal models of parasite burden/infection and antibody levels. The first column gives the response variable for each model and the second column refers to the retained covariates after model reduction. Estimates are given of parameter values once transformed, with 95% confidence intervals in parentheses.

Model response	Final covariates	Estimates	P
<i>Heligmosomoides polygyrus</i> infection	<i>H. polygyrus</i> -specific IgG1 titre	0.37 (0.14 to 0.58)	0.007 <sup>b</sup>
<i>H. polygyrus</i> burden	IgA concentration	−0.02 (−0.03 to −0.01)	0.044 <sup>a</sup>
<i>Eimeria</i> spp. infection	IgA concentration	−0.04 (−0.07 to −0.01)	0.024 <sup>a</sup>
<i>Eimeria</i> spp. burden	NA	NA	NA
Pinworm infection	NA	NA	NA
Pinworm burden	<i>H. polygyrus</i> -specific IgG1 titre	−0.21 (−0.38 to −0.04)	0.023 <sup>a</sup>
IgA concentration	<i>Eimeria</i> spp. infection	−1.77 (−2.91 to −0.62)	0.013 <sup>a</sup>
	Ectoparasite infection	−1.36 (−2.26 to −0.47)	0.014 <sup>a</sup>
<i>H. polygyrus</i> -specific IgG1 titre	<i>Eimeria</i> spp. infection	−2.08 (−3.54 to −0.64)	0.020 <sup>a</sup>
	<i>H. polygyrus</i> infection	2.36 (1.20 to 3.51)	0.001 <sup>b</sup>

$P < 0.05$  was considered significant.

<sup>a</sup>  $P < 0.05$ .

<sup>b</sup>  $P < 0.005$ .

### 3.2. Does parasite infection predict antibody levels?

*Heligmosomoides polygyrus*-specific IgG1 titre was positively associated with *H. polygyrus* infection, and negatively with *Eimeria* spp. infection (Table 1, Fig. 2A), with no other demographic or coinfection terms retained after model selection. The concentration of total faecal IgA was not associated with *H. polygyrus* infection status, but as in the case of *H. polygyrus*-specific IgG1, was negatively associated with *Eimeria* spp. infection status. Furthermore, infection with ectoparasites was also negatively associated with IgA concentration (Table 1, Fig. 2B).

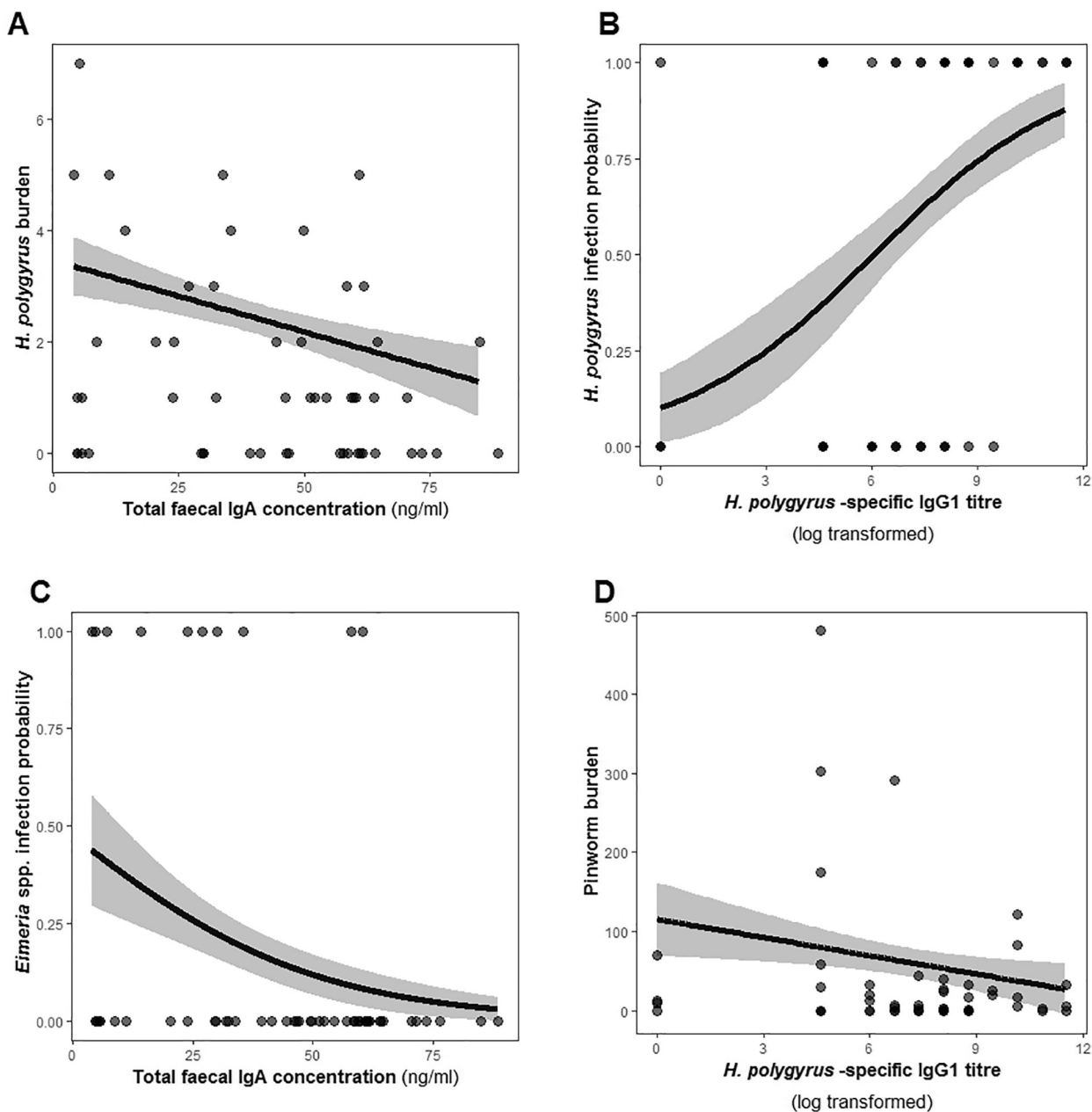
## 4. Discussion

In this study, we used wild wood mice to investigate the inter-relationships between the host's immune response, host demography and natural infection and coinfection. We found that specific and general antibody levels were the strongest predictors of *H. polygyrus*, *Eimeria* spp. and pinworm infection, even given natural variation in host demographic parameters. Interestingly, the presence of *Eimeria* spp. infection within the host was associated with lower levels of both specific and general antibodies, irrespective of helminth or pinworm infection. This could suggest a negative effect of *Eimeria* spp. on the antibody-dependent component of the host's immune response, thereby hinting towards a (partially) immune-mediated interaction between the two parasites. Overall our results highlight how measuring antibody levels in wild animal populations can be crucial to uncover the variable relationships between parasite coinfection and the host's immune response.

Through a drug treatment perturbation experiment, we have previously established a negative interaction between *H. polygyrus* and *Eimeria hungaryensis* in this study system (Knowles et al., 2013), although the mechanism underlying this interaction could not be determined due to a lack of immunological measures. This study provides the first hint towards an involvement of the host's immune response in this interaction, as the presence of *Eimeria* spp. was associated with lower *H. polygyrus*-specific IgG1 antibody. Even though there was only a low number of coinfecting mice in our study, the fact that we observed lower *H. polygyrus*-specific IgG1 whenever *Eimeria* spp. were present in an animal (which includes both coinfecting and *Eimeria* spp. only infected mice), suggests that the effect of *Eimeria* spp. on the host's immune response is more systemic than local and potentially involves a switch from a Th2 to a more mixed Th1/Th2 type response upon *Eimeria* spp.

infection. However, as this study did not involve any anthelmintic treatment, we cannot assess the effect of helminth removal on antibodies and if those are correlated with the previously observed increase in coccidian burden (Knowles et al., 2013). Hence, low initial antibody levels could also have led to an increase in susceptibility towards *Eimeria* spp. Further, there has also been evidence that immunological interference during coinfection between *Eimeria* spp. and *H. polygyrus* can act in the other direction, i.e. nematode infection negatively affects anti-*Eimeria* immune responses (Rausch et al., 2010). Interestingly, *Eimeria vermiformis* replication in laboratory mice has previously been shown to increase during the acute phase of *H. polygyrus* infection, which was correlated with a reduction in pro-inflammatory cytokines such as IFN- $\gamma$ , IL-12 and IL-23, whereas there was no effect of coinfection on coccidian replication during the chronic phase of *H. polygyrus* infection (Rausch et al., 2010). This study highlights the importance of timing of infection in order for different immune pathways to interfere with each other, a factor that is difficult to control for in a wild model system.

In our analysis, we discriminated between the process(es) responsible for parasite infection probability, and parasite burden. In doing so, we found that *H. polygyrus* infection probability was positively associated with *H. polygyrus*-specific IgG1 levels. From laboratory studies, we know that high levels of non-affinity matured and therefore non-parasite-specific IgG and IgE isotypes are produced upon primary *H. polygyrus* infection (McCoy et al., 2008). Parasite-specific antibodies (such as *H. polygyrus*-specific IgG1) are detectable only at approximately 10 days post-primary infection, with their levels increasing much faster on secondary infection (McCoy et al., 2008). Therefore, the positive relationship we observed here between *H. polygyrus*-specific IgG1 and *H. polygyrus* infection likely reflects previous *H. polygyrus* exposure, as no such relationship would be expected in mice upon primary infection. Hence, in the wild, IgG1 can act as an immune marker (i.e., increasing in response to parasite challenge; hence the observed positive relationships with *H. polygyrus* reported here), and it is difficult to determine the extent to which it is important in *H. polygyrus* worm clearance or resistance. However, we know from previous studies that IgG1 reduces female worm fecundity, rather than promoting parasite expulsion (McCoy et al., 2008; Hewitson et al., 2011), meaning it would be unlikely, on an individual level, to observe a negative relationship between IgG1 levels and *H. polygyrus* infection risk or burden, as it likely acts on egg output rather than worm survival. However, at a population level, such a relationship could occur due to reduced egg output due to



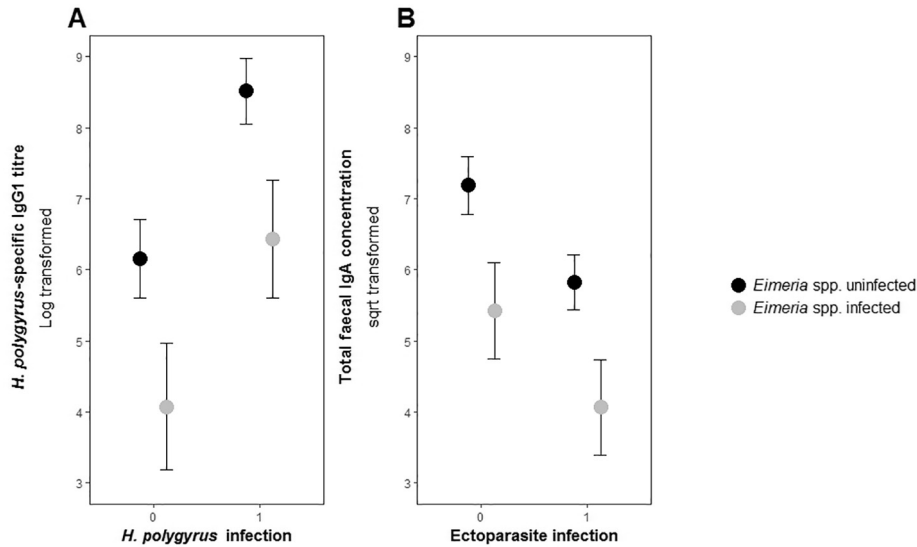
**Fig. 1.** Predicted relationships between antibody levels and parasite infection probability and burden. (A) Negative relationship between total faecal IgA concentration and *Heligmosomoides polygyrus* burden, (B) positive relationship between *H. polygyrus*-specific IgG1 titre and *H. polygyrus* infection probability, (C) negative relationship between total faecal IgA concentrations and *Eimeria* spp. infection probability, and (D) negative relationship between *H. polygyrus*-specific IgG1 titre and pinworm burden. Black lines represent model predictions, grey shaded areas represent S.E. of model predictions.

the action of IgG1. Interestingly, we also found a negative relationship between *H. polygyrus*-specific IgG1 and pinworm burdens. This could reflect correlated exposure of mice to these two helminth species. However, pinworms have a much more direct life-cycle (eggs shed into the environment are directly infective compared with the need for L3 development for *H. polygyrus*) (Scott, 1988), and hence correlated exposure might not lead to correlated infections. As we saw no correlations between *H. polygyrus* burden and pinworm burden (data not shown), we suggest that this could occur through cross-reactivity of IgG1 with pinworms, as previously shown between *H. polygyrus* and *Trichinella spiralis* (Bell et al., 1992).

Our results also suggest a possible protective effect of faecal IgA against high burdens of *H. polygyrus* (Fig. 1A). A similar negative association between helminths and faecal IgA has been found in

wild Soay sheep, where *Teladorsagia circumcincta*-specific IgA was negatively correlated with *T. circumcincta* faecal egg counts (Watt et al., 2016). The same authors also found a positive correlation between total IgA and *T. circumcincta*-specific IgA in faeces. Parasite-specific IgA and IgG1 levels circulating in serum have been shown to reduce survival and fecundity of the helminth *Strongyloides ratti* in infected rats (Bleay et al., 2007). These studies, together with our results, indicate that faecal IgA potentially serves as an important immune effector during natural helminth infections. The mechanisms underlying this effector function could either be a direct negative effect of IgA on *H. polygyrus*, or a protective effect of high IgA levels towards helminth infections.

We further found negative associations between faecal IgA and the presence of both *Eimeria* spp. and ectoparasites. Ectoparasites are known to be vectors for a number of other microparasitic



**Fig. 2.** Predicted relationships between parasitic infection and antibody levels. (A) While *Heligmosomoides polygyrus*-infected mice had higher *H. polygyrus*-specific IgG1 titres, the presence of *Eimeria* spp. lead to lower worm-specific antibodies. (B) We found a negative relationship between ectoparasitic infection and total faecal IgA, and similarly found that *Eimeria* spp.-infected mice had lower IgA antibody levels. Points represent predicted means  $\pm$  S.E.

infections in this system such as *Bartonella* spp. (flea-transmitted; (Telfer et al., 2008), *Babesia* spp. and *Borrelia* spp. (tick-transmitted; (Burri et al., 2014; Obiegala et al., 2015)). Further, ectoparasites by themselves can also lead to elevated levels of the innate immune system (Jackson et al., 2009; Rynkiewicz et al., 2013). However, since we did not test for the presence of any microparasites other than *Eimeria* spp., we cannot determine whether microparasitic infection due to ectoparasitic infection accounts for the relationship between ectoparasitic infection and IgA levels (i.e., whether ectoparasitic infection is a proxy for microparasitic infection). In the case of *Eimeria* spp. infections, rats and chickens have both shown elevated intestinal IgA levels following infection with *Eimeria nieschulzi* (Smith et al., 1995) and *Eimeria tenella* (Trees et al., 1989), respectively. Since the role of IgA in anti-*Eimeria* immune response is not entirely clear (Lillehoj and Trout, 1994), we cannot definitively determine the direction of cause-and-effect for the negative associations between total faecal IgA and *Eimeria* spp. Therefore, it is possible that *Eimeria* spp. reduce IgA levels (immunosuppressive effect or switch from Th2 to Th1 immune response), or that pre-established high concentrations of IgA antibodies protected mice against *Eimeria* spp. (protection effect).

Overall, our study shows that the effect of antibodies on parasitic infections was independent of variation in host demographic factors such as sex, age or body mass. Since mice can be assumed to have experienced various parasitic challenges over the course of their lives, this unknown variation in infection history can make it difficult to allocate cause-and-effect relationships in cross-sectional data on immune measurements. However, we showed that antibody levels can be a valuable measure in order to identify individuals within a population that are either at risk of acquiring parasitic infection, or have experienced parasitic infection in the past. Future work involving a greater number of animals might increase the resolution for some demographic factors tested here and hence increase statistical power for detection of associations between parasitic infection and host demographics. Further, investigating the cause-and-effect relationship between antibody levels, parasitic infection and host health will increase our understanding of the role of the antibodies measured here during natural parasitic infection, as well as their significance as a diagnostic tool in cross-sectional surveys of host health.

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## References

- Babayan, S.A., Allen, J.E., Bradley, J.E., Geuking, M.B., Graham, A.L., Grencis, R.K., Kaufmann, J., McCoy, K.D., Paterson, S., Smith, K.G.C., Turnbaugh, P.J., Viney, M. E., Maizels, R.M., Pedersen, A.B., 2011. Wild immunology: converging on the real world. *Ann. N.Y. Acad. Sci.* 1236, 17–29.
- Behnke, J.M., Lowe, A., Clifford, S., Wakelin, D., 2003. Cellular and serological responses in resistant and susceptible mice exposed to repeated infection with *Heligmosomoides polygyrus bakeri*. *Parasite Immunol.* 25, 333–340.
- Bell, R.G., Appleton, J.A., Negrao-Correa, D.A., Adams, L.S., 1992. Rapid expulsion of *Trichinella spiralis* in adult rats mediated by monoclonal antibodies of distinct IgG isotypes. *Immunology* 75, 520–527.
- Ben-Smith, A., Wahid, F.N., Lamm, D.A., Behnke, J.M., 1999. The relationship between circulating and intestinal *Heligmosomoides polygyrus*-specific IgG1 and IgA and resistance to primary infection. *Parasite Immunol.* 21, 383–395.
- Beura, L.K., Hamilton, S.E., Bi, K., Schenkel, J.M., Odumade, O.A., Casey, K.A., Thompson, E.A., Fraser, K.A., Rosato, P.C., Filali-Mouhim, A., Sekaly, R.P., Jenkins, M.K., Vezys, V., Haining, W.N., Jameson, S.C., Masopust, D., 2016. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* 532, 512–516.
- Bleay, C., Wilkes, C.P., Paterson, S., Viney, M.E., 2007. Density-dependent immune responses against the gastrointestinal nematode *Strongyloides ratti*. *Int. J. Parasitol.* 37, 1501–1509.
- Burlet, P., Deplazes, P., Hegglin, D., 2010. Efficient age determination: how freezing affects eye lens weight of the small rodent species *Arvicola terrestris*. *Eur. J. Wildl. Res.* 56, 685–688.
- Burri, C., Schumann, O., Schumann, C., Gern, L., 2014. Are *Apodemus* spp. mice and *Myodes glareolus* reservoirs for *Borrelia miyamotoi*, *Candidatus Neorickettsia mikurensis*, *Rickettsia helvetica*, *R. monacensis* and *Anaplasma phagocytophilum*? *Ticks Tick Borne Dis.* 5, 245–251.
- Burthe, S., Telfer, S., Lambin, X., Bennett, M., Carslake, D., Smith, A., Begon, M., 2006. Cowpox virus infection in natural field vole *Microtus agrestis* populations: delayed density dependence and individual risk. *J. Anim. Ecol.* 75, 1416–1425.



- Charlier, J., Velde, F.V., van der Voort, M., Van Meensel, J., Lauwers, L., Cauberghe, V., Vercruyse, J., Claerebout, E., 2015. ECONOHEALTH: Placing helminth infections of livestock in an economic and social context. *Vet. Parasitol.* 212, 62–67.
- Charmantier, A., Garant, D., 2005. Environmental quality and evolutionary potential: lessons from wild populations. *Proc. Biol. Sci.* 272, 1415–1425.
- Chipeta, M.G., Ngwira, B.M., Simoonga, C., Kazembe, L.N., 2014. Zero adjusted models with applications to analysing helminths count data. *BMC Res. Notes* 7, 856.
- Christensen, N.Ø., Nansen, P., Fagbemi, B.O., Monrad, J., 1987. Heterologous antagonistic and synergistic interactions between helminths and between helminths and protozoans in concurrent experimental infection of mammalian hosts. *Parasitol. Res.* 73, 387–410.
- Cox, F.E.G., 2001. Concomitant infections, parasites and immune responses. *Parasitology* 122, S23–S38.
- Davis, P.J., Parry, S.H., Porter, P., 1978. The role of secretory IgA in anti-coccidial immunity in the chicken. *Immunology* 34, 879–888.
- Downs, J.A., Dupnik, K.M., van Dam, G.J., Urassa, M., Lutonja, P., Kornelis, D., de Dood, C.J., Hoekstra, P., Kanjala, C., Isingo, R., Peck, R.N., Lee, M.H., Corstjens, P., Todd, J., Chagalucha, J.M., Johnson Jr., W.D., Fitzgerald, D.W., 2017. Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load at HIV-1 seroconversion: A nested case-control study. *PLoS Negl. Trop. Dis.* 11, e0005968.
- Dryden, M.W., Payne, P.A., Ridley, R., Smith, V., 2005. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Vet. Ther.* 6, 15–28.
- Ezenwa, V.O., Etienne, R.S., Luikart, G., Beja-Pereira, A., Jolles, A.E., 2010. Hidden consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African Buffalo. *Am. Nat.* 176, 613–624.
- Fagarasan, S., Honjo, T., 2003. Intestinal IgA synthesis: regulation of front-line body defences. *Nat. Rev. Immunol.* 3, 63–72.
- Friberg, I.M., Lowe, A., Ralli, C., Bradley, J.E., Jackson, J.A., 2011. Temporal anomalies in immunological gene expression in a time series of wild mice: signature of an epidemic? *PLoS One* 6, e20070.
- Grear, D.A., Hudson, P., 2011. The dynamics of macroparasite host-self-infection: a study of the patterns and processes of pinworm (*Oxyuridae*) aggregation. *Parasitology* 138, 619–627.
- Griffiths, E.C., Pedersen, A.B., Fenton, A., Petchey, O.L., 2011. The nature and consequences of coinfection in humans. *J. Infect.* 63, 200–206.
- Griffiths, E.C., Pedersen, A.B., Fenton, A., Petchey, O.L., 2014. Analysis of a summary network of co-infection in humans reveals that parasites interact most via shared resources. *Proc. Biol. Sci.* 281, 20132286.
- Gulland, F.M.D., 1992. The role of nematode parasites in Soay sheep (*Ovis aries* L.) mortality during a population crash. *Parasitology* 105, 493–503.
- Hewitson, J.P., Filbey, K.J., Grainger, J.R., Dowle, A.A., Pearson, M., Murray, J., Hargus, Y., Maizels, R.M., 2011. *Heligmosomoides polygyrus* elicits a dominant nonprotective antibody response directed against restricted glycan and peptide epitopes. *J. Immunol.* 187, 4764–4777.
- Hudson, P.J., Dobson, A.P., Newborn, D., 1998. Prevention of population cycles by parasite removal. *Science* 282, 2256–2258.
- Jackson, J.A., Friberg, I.M., Bolch, L., Lowe, A., Ralli, C., Harris, P.D., Behnke, J.M., Bradley, J.E., 2009. Immunomodulatory parasites and toll-like receptor-mediated tumour necrosis factor alpha responsiveness in wild mammals. *BMC Biol.* 7, 16.
- Japp, A.S., Hoffmann, K., Schlickeiser, S., Glauben, R., Nikolaou, C., Maecker, H.T., Braun, J., Matzmohr, N., Sawitzki, B., Siegmund, B., Radbruch, A., Volk, H.D., Frentsch, M., Kunkel, D., Thiel, A., 2017. Wild immunology assessed by multidimensional mass cytometry. *Cytometry A* 91, 85–95.
- Knowles, S.C., Fenton, A., Petchey, O.L., Jones, T.R., Barber, R., Pedersen, A.B., 2013. Stability of within-host-parasite communities in a wild mammal system. *Proc. Biol. Sci.* 280, 20130598.
- Lang, R., Schick, J., 2017. Review: Impact of Helminth Infection on Antimycobacterial Immunity—A Focus on the Macrophage. *Front. Immunol.* 8, 1864.
- Lillehoj, H.S., Trout, J.M., 1994. CD8+ T Cell-Coccidia Interactions. *Parasitol Today* 10.
- Macpherson, A.J., Geuking, M.B., McCoy, K.D., 2012. Homeland security: IgA immunity at the frontiers of the body. *Trends Immunol.* 33, 160–167.
- Maizels, R.M., Hewitson, J.P., Murray, J., Hargus, Y.M., Dayer, B., Filbey, K.J., Grainger, J.R., McSorley, H.J., Reynolds, L.A., Smith, K.A., 2012. Immune modulation and modulators in *Heligmosomoides polygyrus* infection. *Exp. Parasitol.* 132, 76–89.
- McCoy, K.D., Stoel, M., Stettler, R., Merky, P., Fink, K., Senn, B.M., Schaer, C., Massacand, J., Odermatt, B., Oettgen, H.C., Zinkernagel, R.M., Bos, N.A., Hengartner, Macpherson, A.J., Harris, N.L., 2008. Polyclonal and specific antibodies mediate protective immunity against enteric helminth infection. *Cell Host Microbe* 4, 362–373.
- Nussey, D.H., Watt, K.A., Pilkington, J.G., Zamojska, R., McNeilly, T.N., 2012. Age-related variation in immunity in a wild mammal population. *Aging Cell* 11, 178–180.
- Obiegala, A., Pfeffer, M., Pfister, K., Karnath, C., Silaghi, C., 2015. Molecular examinations of *Babesia microti* in rodents and rodent-attached ticks from urban and sylvatic habitats in Germany. *Ticks Tick Borne Dis.* 6, 445–449.
- Pedersen, A.B., Babayan, S.A., 2011. Wild immunology. *Mol. Ecol.* 20, 872–880.
- Pedersen, A.B., Fenton, A., 2007. Emphasizing the ecology in parasite community ecology. *Trends Ecol. Evol.* 22, 133–139.
- Pedersen, A.B., Greives, T.J., 2008. The interaction of parasites and resources cause crashes in a wild mouse population. *J. Anim. Ecol.* 77, 370–377.
- Petney, T.N., Andrews, R.H., 1998. Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. *Int. J. Parasitol.* 28, 377–393.
- Poulin, R., 2013. Explaining variability in parasite aggregation levels among host samples. *Parasitology* 140, 541–546.
- Rausch, S., Held, J., Stange, J., Lendner, M., Hepworth, M.R., Klotz, C., Lucius, R., Pogonka, T., Hartmann, S., 2010. A matter of timing: early, not chronic phase intestinal nematode infection restrains control of a concurrent enteric protozoan infection. *Eur. J. Immunol.* 40, 2804–2815.
- Reynolds, L.A., Filbey, K.J., Maizels, R.M., 2012. Immunity to the model intestinal helminth parasite *Heligmosomoides polygyrus*. *Semin. Immunopathol.* 34, 829–846.
- Rigaud, T., Perrot-Minnot, M.J., Brown, M.J., 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proc. Biol. Sci.* 277, 3693–3702.
- Rose, H., Hoar, B., Kutz, S.J., Morgan, E.R., 2014. Exploiting parallels between livestock and wildlife: Predicting the impact of climate change on gastrointestinal nematodes in ruminants. *Int. J. Parasitol. Parasites. Wildl.* 3, 209–219.
- Rowe, F.P., Bradfield, A., Quay, R.J., Swinney, T., 1985. Relationship between eye lens weight and age in the wild house mouse (*Mus musculus*). *J. Appl. Ecol.* 22, 55–61.
- Rynkiewicz, E.C., Hawlena, H., Durden, L.A., Hastriter, M.W., Demas, G.E., Clay, K., 2013. Associations between innate immune function and ectoparasites in wild rodent hosts. *Parasitol. Res.* 112, 1763–1770.
- Scott, M.E., 1988. Predisposition of Mice to *Heligmosomoides polygyrus* and *Aspiculuris tetraptera* (Nematoda). *Parasitology* 97, 101–114.
- Smith, N.C., Ovington, K.S., Deplazes, P., Eckert, J., 1995. Cytokine and immunoglobulin subclass responses of rats to infection with *Eimeria nieschulzi*. *Parasitology* 11, 51–57.
- Sorobetea, D., Svensson-Frej, M., Grecis, R., 2018. Immunity to gastrointestinal nematode infections. *Mucosal Immunol.* 11, 304–315.
- Stephenson, L.S., Latham, M.C., Ottesen, E.A., 2000. Malnutrition and parasitic helminth infections. *Parasitology* 121, S23–S38.
- Taffs, L.F., 1976. Pinworm infections in laboratory rodents: a review. *Lab. Anim.* 10, 1–13.
- Tanikawa, T., 1993. An eye-lens weight curve for determining age in black rats, *Rattus rattus*. *J. Mammal. Soc. Jpn.* 18, 49–51.
- Telfer, S., Birtles, R., Bennett, M., Lambin, X., Paterson, S., Begon, M., 2008. Parasite interactions in natural populations: insights from longitudinal data. *Parasitology* 135, 767–781.
- Trees, A.J., Karim, M.J., McKellar, S.B., Carter, S.D., 1989. *Eimeria tenella*: local antibodies and interactions with the sporozoite surface. *J. Protozool.* 36, 326–333.
- Turner, A.K., Begon, M., Jackson, J.A., Bradley, J.E., Paterson, S., 2011. Genetic diversity in cytokines associated with immune variation and resistance to multiple pathogens in a natural rodent population. *PLoS Genet.* 7, e1002343.
- Viney, M., Riley, E.M., 2017. The immunology of wild rodents: current status and future prospects. *Front. Immunol.* 8, 1481.
- Watt, K.A., Nussey, D.H., Maclellan, R., Pilkington, J.G., McNeilly, T.N., 2016. Fecal antibody levels as a noninvasive method for measuring immunity to gastrointestinal nematodes in ecological studies. *Ecol. Evol.* 6, 56–67.
- Weinstein, S.B., Lafferty, K.D., 2015. How do humans affect wildlife nematodes? *Trends Parasitol.* 31, 222–227.
- Withenshaw, S.M., Devevey, G., Pedersen, A.B., Fenton, A., 2016. Multihost *Bartonella* parasites display covert host specificity even when transmitted by generalist vectors. *J. Anim. Ecol.* 85, 1442–1452.